PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 050049wo/Me/do	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2005/000301	International filing date *(day/month/year)* 14.01.2005	Priority date *(day/month/year)* 15.01.2004
International Patent Classification (IPC) or national classification and IPC G01N33/68		
Applicant EVOTEC AG		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 8 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 5 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☒	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14.11.2005	12.12.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Jenkins, G Telephone No. +31 70 340-2608

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2005/000301

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-19 as originally filed

Claims, Numbers

1-29 received on 15.11.2005 with letter of 14.11.2005

Drawings, Sheets

1/5-5/5 as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 30,31
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-31 [w.r.t. industrial applicability]

because:

☒ the said international application, or the said claims Nos. 1-31 [w.r.t. industrial applicability] relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-29
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-29
Industrial applicability (IA)	Yes:	Claims	No opinion
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to industrial applicability

1 The subject-matter of independent claim 1 contains the step "providing a sample comprising ion channels". It is clear from dependent claim 4 that the sample can comprise human or animal cells. Thus, the subject-matter of independent claim 1, and that of claims 2-29 dependent thereon, encompasses both a method of surgery and a method of diagnosis practised on the human body covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2 Reference is made to the following documents:

D1: SIEM-FUNG D J ET AL: "THE EFFECT OF TEMPERATURE ON VERATRIDINE ACTION IN SQUID GIANT AXONS" BIOCHIMICA ET BIOPHYSICA ACTA, vol. 728, no. 3, 1983, pages 305-310, XP008045560 ISSN: 0006-3002

D2: KIM C S ET AL: "Voltage-dependent calcium channels in ventricular cells of rainbow trout: effect of temperature changes in vitro." AMERICAN JOURNAL OF PHYSIOLOGY. REGULATORY, INTEGRATIVE AND COMPARATIVE PHYSIOLOGY. JUN 2000, vol. 278, no. 6, June 2000 (2000-06), pages R1524-R1534, XP002324413 ISSN: 0363-6119

D3: MITSUIYE TAMOTSU ET AL: "Temperature dependence of the inward rectifier K+ channel gating in guinea-pig ventricular cells" JAPANESE JOURNAL OF PHYSIOLOGY, vol. 47, no. 1, 1997, pages 73-79, XP002324414 ISSN: 0021-521X

D4: CHUNG SHIN-HO ET AL: "Changes in the kinetics and conductance of N-methyl-D-aspartate (NMDA)-receptor activated single channels with temperature" NEUROSCIENCE LETTERS, vol. 187, no. 3, 1995, pages 181-184, XP002324415 ISSN: 0304-3940

D5: DING J P ET AL: "Modulation of mechanosensitive calcium-selective cation channels by temperature." THE PLANT JOURNAL : FOR CELL AND MOLECULAR BIOLOGY. MAY 1993, vol. 3, no. 5, May 1993 (1993-05), pages

713-720, XP002324416 ISSN: 0960-7412

D6: NETZER RAINER ET AL: "Screening lead compounds for QT interval prolongation" DRUG DISCOVERY TODAY, ELSEVIER SCIENCE LTD, GB, vol. 6, no. 2, January 2001 (2001-01), pages 78-84, XP002198162 ISSN: 1359-6446

D7: NUMANN R ET AL: "High-throughput screening strategies for cardiac ion channels" TRENDS IN CARDIOVASCULAR MEDICINE 2001 UNITED STATES, vol. 11, no. 2, 2001, pages 54-59, XP002324417 ISSN: 1050-1738

3 NOVELTY AND INVENTIVE STEP

3.1 D1 discloses: a method of determining the effects of veratridine on ion channel activity in squid axons, by measuring the cell membrane potential and ion (potassium and sodium) concentration of the said squid axons, wherein the said method is carried out at 5°C (figure 1, tables 1 and 2).

3.2 D2 discloses: a method of determining the effects of forksolin and Bay K8644 on voltage-gated calcium ion channel activity in ventricular cells by measuring the cell membrane potential, wherein the said method is carried out at 4°C (figures 9 and 10, tables 1 and 2).

3.3 D3 discloses: a method of measuring the activity of voltage-sensitive potassium channels of cardiac myocytes, wherein the membrane potential of is determined at 5°C (figure 2).

3.4 D4 discloses: a method of measuring the activity of transmitter-dependent ion channels of hippocampal cells, wherein the membrane potential is determined at 5°C (figure 1).

3.5 D5 discloses: a method of measuring the activity of mechano-sensitive calcium channels of onion epidermal cells, wherein the ion channel currents are determined at 1-5°C (p. 713, column 2, paragraph 3; figure 1).

3.6 D6 and D7 show that the use of voltage-sensitive or ion-sensitive fluorescent indicators in screening assays for modulators of ion channels is common general

knowledge (D6, p. 82; D7, p. 56, column 2 - p. 57, column 2).

3.7 In light of D1-D7, the subject-matter of claims 1-29 is novel (Article 33(2) PCT). None of these documents disclose the following combination of features 1) temperature < or = 10°C, 2) fluorescence or radioactive or atomic absorption spectroscopy methods, 3) measuring membrane potentials or ion concentrations.

3.8 With respect to claim 1, D1 is considered the closest prior art.

3.8.1 The additional technical feature of claim 1 over D1 is the replacement of the patch clamp for fluorescence or radioactive or atomic absorption spectroscopy methods to measure membrane potentials or ion concentrations (i.e. activity of ion chanels).

3.8.2 The application and examples do not establish any technical effect associated with this modification.

3.8.3 The objective technical problem is therefore the provision of an alternative means to the patch clamp for measuring membrane potentials or ion concentrations in the method of D1.

3.8.4 The solution is provided by the subject-matter of claim 1 through the replacement of the patch clamp for fluorescence or radioactive or atomic absorption spectroscopy methods.

3.8.5 However, fluorescent methods are well-known equivalents to the patch clamp for measuring membrane potentials or ion concentrations. Indeed review article D6 can be considered to represent **common general knowledge in the art** at the filing date of the present application. It lists a variety of **standard** methods for measuring ion channel activities among which are patch clamp / electrophysiology methods (table 3) and fluorescent methods (p. 82). D7 also discloses fluorescent methods as a **standard** way for measuring ion channel activities. Thus, it would have been obvious to solve the problem posed by replacing the patch clamp in D1 for fluorescence or radioactive or atomic absorption spectroscopy methods. Therefore, the subject-matter of claim 1 is not inventive (Article 33(3) PCT).

3.9 In light of documents D1-D7 either alone or in combination, and the technical disclosure of the present application, the dependent claims are either not new (Article

33(2) PCT) or not inventive (Article 33(3) PCT).

3.10 Notwithstanding the above, it is clear from the description that the alleged invention depends on obtaining the following technical effect: a reduction in bias when monitoring the influence of a potential or known pharmacologically active substance. However, independent claim 1 does not contain a feature corresponding to "monitoring the effects of a potential or known pharmacologically active substance". Thus, independent claim 1 lacks features that are essential for producing the desired technical effect - a *conditio sine qua non* for acknowledging the presence of an inventive step (Article 33(3) PCT).

Re Item VII

Certain defects in the international application

4 Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in D1 and D2 is not mentioned in the description.

Re Item VIII

Certain observations on the international application

5 The approximate term "about" used in claims 1-3 has no precise meaning within the art, rendering the scope of the said claims uncertain. Therefore, the subject-matter of claims 1-29 does not comply with the requirements of Article 6 PCT.

AMENDED CLAIMS (fair copy)

1. A method for examining the activity of ion channels, comprising the following steps:

   - providing a sample comprising ion channels; and

   - determining a value of a measuring parameter as an indicator of the activity of the ion channels, the measuring parameter being a membrane potential, a measure of a membrane potential, an ion concentration, or a measure of an ion concentration;

   characterised in that said determining of the value of the measuring parameter is performed at a temperature of ≤ about 10 °C by fluorescence methods, radioactive methods or atomic absorption spectroscopy.

2. The method according to claim 1, characterized in that said determining of the value of the measuring parameter is performed at a temperature of ≤ about 5 °C, especially ≤ about 2 °C.

3. The method according to claim 1 or 2, characterized in that said determining of the value of the measuring parameter is performed at a temperature of from about 10 °C to –4 °C, especially from about 5 °C to -4 °C, more preferably from about 5 °C to 0 °C, even more preferably from about 2 °C to 0 °C.

4. The method according to any of the preceding claims, characterized in that the sample comprises one or more cells or cell organelles which have ion channels, in particular human or animal cells or cell organelles.

5. The method according to any of the preceding claims, characterized in that the sample comprises one or more vesicles which have ion channels.

6. The method according to any of the preceding claims, characterized in that the sample comprises membrane bound ion channels, in particular ion channels embedded into a membrane of cells, cell organelles, vesicles or embedded into an artificial membrane.

7. The method according to any of the preceding claims, characterized in that said measuring parameter is the membrane potential of a cell, cell organelle or vesicle, or a measure of said membrane potential.

8. The method according to any of the preceding claims, characterized in that the measuring parameter is an extracellular, intracellular, extravesicular and/or intravesicular ion concentration or a measure thereof.

9. The method according to any of the preceding claims, characterized in that the value of said measuring parameter is determined before, during and/or after the addition of a test substance which potentially influences the activity of the ion channels.

10. The method according to any of the preceding claims, characterized in that the activity of a transmitter-dependent ion channel is examined.

11. The method according to any of the preceding claims, characterized in that the activity of a voltage-sensitive ion channel is examined.

12. The method according to any of the preceding claims, characterized in that the activity of a potassium channel, chloride channel, sodium channel or calcium channel is examined.

13. The method according to any of the preceding claims characterized in that an optical response of (i) a carbocyanine derivative, in particular a thia-, indo-, or oxa-carbocyanine or an iodide derivative of a carbocyanine, (ii) a

rhodamine dye, (iii) an oxonol dye, (iv) merocyanine 540, or (v) a styryl dye serves as a measure of the membrane potential.

14. The method according to any of the preceding claims, characterized in that the fluorescence emission of a voltage-sensitive fluorescent dye, preferably a DiBAC dye, more preferably the dye $Dibac_4(3)$, serves as a measure of the membrane potential.

15. The method according to any of the preceding claims, characterized in that the ion concentration of rubidium, especially of non-radioactive rubidium, is determined as an indicator of the activity of the ion channels.

16. The method according to any of the preceding claims, characterized in that the ion concentration, especially the ion concentration of calcium, is measured by means of chelating agents.

17. The method according to any of the preceding claims, characterized in that the values of several measuring parameters are determined.

18. The method according to any of the preceding claims for use in the research on pharmaceutically active substances, especially in the medium- or high-throughput screening of potentially or established active pharmaceutical substances, in particular the identification of potentially active pharmaceutical substances or the determination of side effects of potentially or established active pharmaceutical substances.

19. The method according to any of the preceding claims for use in the agricultural research, especially in the research on agrochemicals as e.g. insectizids.

20. Use of a voltage-sensitive or ion-sensitive indicator for the conductance of the method according to any of the preceding claims.

21. Use according to claim 20 wherein the ion-sensitive indicator is a calcium indicator, in particular a fluo-calcium indicator, a fura indicator, an indo indicator, Calcium Green™, or Oregon Green™.

22. Use according to claim 20 wherein the ion-sensitive indicator is a sodium or potassium indicator, preferably a fluorescent sodium or potassium indicator, in particular SBFI, PBFI, Sodium Green Na^+ indicator, CoroNa Green Na^+ indicator, or CoroNa Red Na^+ indicator.

23. Use according to claim 20 wherein the voltage-sensitive indicator is a carbocyanine derivative, in particular an indo-, thia-, or oxa- carbocyanine or a iodide derivative of a carbocyanine; a rhodamine dye; an oxonol dye; merocyanine 540; or a styryl dye.

24. Use according to claim 23 wherein the oxonol dye is a bis-isoxazolone oxonol dye or a bis-barbituric acid oxonol (DiBAC) dye, in particular $DiBAC_4(3)$, $DiSBAC_2(3)$ or $DiBAC_4(5)$.

25. Use according to claim 23 wherein the styryl dye is an ANEP (AminoNaphthylEthenylPyridinium) dye, in particular di-4-ANEPPS, di-8-ANEPPS, di-2-ANEPEQ, di-8-ANEPPQ, di-12-ANEPPQ, di-1-ANEPIA, or a dialkylaminophenylpolyenylpyridinium dye (RH dye), in particular RH 414, RH 421, RH 795 or RH 237.

26. Use of a chelating agent for the conductance of the method according to any of claims 1 to 19.

27. Use of rubidium, in particular non-radioactive rubidium, for the conductance of the method according to any of claims 1 to 19.

28. Use of an atomic absorption spectrometer, a flow cytometer, a fluorescence microcope or fluorescence plate reader for the conductance of the method according to any of claims 1 to 19.

29. Use according to claim 28 applying a voltage-sensitive or ion-sensitive indicator according to any of claims 20 to 25, a chelating agent according to claim 26 and/or rubidium according to claim 27.